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VISION BIOSYSTEMS LIMITED

A U S T R A L I A

Patents Act 1990

PROVISIONAL SPECIFICATION

for the invention entitled:

"A COVERTILE FOR A SUBSTRATE"

The invention is described in the following statement:

A COVERTILE FOR A SUBSTRATE

Field of the invention

The present invention relates to a covertile for a substrate.

Background of the invention

The present invention relates to a covertile for a substrate, and in one form a covertile for use with a microscope slide.

Microscope slides are commonly used to view samples of material under a microscope. The samples may contain human tissue, and may require treatment such as staining, so that properties of the sample can be identified. Other materials such as DNA, RNA, or proteins may be included on the slide.

It is common for several reactions to be undertaken on a sample on a slide. Once the reactions have taken place the slide may be viewed under a microscope. Performing the reactions on the slide can be difficult to automate, as the tissue samples require careful preparation and certain reactions require controlled temperatures and/or controlled times.

In some reactions, consistency of result within the sample and/or across a number of samples is important. This type of consistency can be difficult to achieve manually, and requires an experienced operator.

Summary of the Present Invention

In one form, the present invention provides a covertile for a substrate containing a sample, comprising:

a side having a recess;

a fluid receiving zone in fluid communication with the recess; and

a locator such that the covertile may be controlled and located with respect to the substrate.

Preferably the fluid receiving zone is situated at a first end of the covertile.

Preferably the recess is a substantially planar recessed from a land portion.

Preferably the land portion is located at the edge of the covertile, surrounding the recess on two or more sides.

Preferably the locator is situated at a second end of the covertile remote from the first end.

Preferably, the covertile is made from a polymer material.

In one form the recess includes a coating of reduced surface roughness than the polymer material.

In another form the recess includes a coating with reduced porosity.

In another form the recess has one or more coatings.

Preferably a first coating is a material having smilar properties to the material of the slide.

Preferably the first coating is silicon dioxide.

Preferably a second coating is placed intermediate a first coating to provide improved contact properties between the covertile and first coating.

Preferably, the width of the recess of the covertile is no larger than the width of a microscope slide.

In one form the fluid receiving zone includes projections around a fluid-receiving zone.

In another form, two projections form a fluid retention zone at the fluid receiving zone.

Brief Description of the Drawings

Figure 1 shows an example of a typical microscope slide

Figures 2 (a)-(d) shows views of a first example of a covertile;

Figures 3 (a) and (b) show further views of the covertile of figure 2;

Figures 4 (a)-(c) show further views of the covertile in figure 2;

Figure 5 shows a cutaway section of the covertile of figure 2 on the slide shown in figure 1.

Figure 6 shows a schematic cross section of a covertile;

Figure 7 shows a tray adapted to locate covertiles and slides;

Figures 8 (a) and (b) show schematic views of a further example of a covertile nose portion;

Figures 9 (a) and (b) show schematic views of a further example of a covertile nose portion;

Figures 10 (a) and (b) show schematic views of a further example of a covertile nose portion;

Figures 11 (a) and (b) show schematic views of a further example of a covertile nose portion;

Figures 12 (a) and (b) show schematic views of a further example of a covertile nose portion;

Figures 13 (a) and (b) shows schematic views of a further example of a covertile nose portion;

Figures 14 (a) and (b) show views of a further example of a covertile;

Figure 15 shows a further example of a schematic of a covertile nose portion in relation to a covertile;

Figure 16 (a) and (b) show schematic views of a further example of a covertile nose portion;

Figure 17shows a further example of a schematic of a covertile nose portion in relation to a covertile;

Figures 18 (a)-(c) shows the covertile of figure 2 in various positions over a slide of figure 1;

Figure 19 shows the covertile of figure 2 mounted to the tray of figure 7;

In figures 2 (a)-(d) there is shown an example of a covertile 10 having a body 12, a fluid receiving zone 14, a locating means 16 and a recess 18 on face 19. Surrounding the recess 18 on two sides is a land portion 20. Legs 21 extend from the body 12 past a projection 13 to form a fluid retention zone 17.

The covertile 10 is adapted to be used with a microscope slide 1 with an upper surface 2 containing a sample 3, as shown in Figure 1. Each slide 1 is identified by a unique bar code 4. The sample 3, such as a thinly sliced tissue section, is located on the slide 1 in a sample holding region 5.

The recess 18 shown in figure 6 has a generally planar recessed face 22 set in from the land portion 20 by approximately 100 microns. The recess may vary according to application, typically from 20-200 microns. The land portion 20 is adapted to support the covertile on the slide 1. The recessed face 22, land portion 20 and sample holding region 5 of a slide 1 form a reaction chamber 24 when the covertile 10 is placed at least partially over the sample holding region 5.

There are clamping forces applied to the covertile once loaded into the reaction apparatus, and these forces are designed to provide a seal between the land portions 20 and the upper surface of the slide 1. This is to restrict fluid leakage from the side of the covertile. In one example (not shown) the land portions may have an additional member to assist sealing of the land portions with the upper surface 2 of the slide 1. This additional member may be a softer polymer or rubber material.

The fluid retention zone 17 is shown in figure 4 (c), which is a detailed view of the section taken across the covertile 10 and slide shown in figure 4 (a) and (b). The projection 13 has a leg 21 at either end, and is raised to form a volume capable of holding fluid dispensed onto the slide 1. In this way fluid retention zone 17 enables fluid dispensed onto slide 1 to be held until required, without spilling off an edge of the slide. The projection 13 assists in spreading the fluid across the full width of the recess. The fluid retention zone is typically sized to be larger than the volume of the reaction chamber 24, for example 150% of the volume of the reaction chamber. This provides sufficient volume of fluid to fill

the reaction chamber completely, while allowing some excess to flush the chamber, and an amount to be retained in the fluid retention zone to provide a reservoir for evaporation.

The covertile 10 also includes engaging surfaces in the form of wings 26. The wings are adapted to engage ramps 28 on tray 21 thereby lifting the covertile clear of the surface of the slide 1. An example of the wings lifting the covertile free is shown in figure 7 and 19. The covertile 10 may be controlled by an arm (not shown) moving the locating means 16. The covertile 10 may be placed in a number of positions over the slide, exemplified by the positions of the covertile relative to the slide shown in figure 18. In figure 18(a), the covertile is in an open position relative to the slide, as the sample is exposed and open. Figure 18 (b) shows the covertile in a partially closed position, and figure 18 (c) shows the covertile in a fully closed position, where the sample is completely covered by the covertile and is therefore wholly contained within the reaction chamber 24. The reaction chamber formed by the covertile and recess 18 as shown in figure 5 extends over most of the slide 1. However it is possible that the sample may be placed more towards the end of the slide distal from the bar code, and therefore a smaller reaction chamber is required. Reducing the size of the reaction chamber reduces the amount of fluid required to fill the chamber, which can be important where expensive or scarce fluids are used. It is possible to form a smaller reaction chamber with a standard sized covertile, by only covering a porion of the slide 1 with the covertile 10. This position is shown in figure 18 (b).

Variations in covertile arrangement are schematically shown in figures 8-17. In figures 8-17, only the front segments of the covertiles are shown, and the locating means have been omitted from view.

In figure 8(a) a covertile 50 is shown having a body 51, projecting legs 52, a protruding section 54 and an indent 56. The projecting legs 50 either side of the body 51 form a fluid receiving zone 58. When placed onto a slide, fluid may be dispensed into the fluid receiving zone, where it spreads in a circular fashion to contact the protruding section 54. The indent 56 allows the fluid to contact a

wider portion of the protruding section 54 than if the front edge of the protruding section was straight (as shown in figure 9). Once the fluid is in contact with the protruding section, it wicks across the width of the recess 18. If suction is applied at the rear of the recess, or the covertile is moved along the slide from an open position to a more closed position, then the fluid begins to fill the recess 18. When the recess has moved across the sample 3, it forms the reaction chamber 24 as the fluid may react with the sample 3.

Figures 9 (a) and (b) show a more simple construction of a covertile 60 that may be used in some circumstances. The operation of the covertile 60 is the same as the operation of the covertile 50 in figures 8 (a) and (b).

Figure 10s (a) and (b) show covertile 70 having a body 71 with projecting legs 72. A protruding section 74 and a bar 75 surround a fluid receiving zone 78 for receiving fluid. The fluid may be dispensed onto the protrusion, where it flows down the protrusion and onto the slide surface. The protrusion 74 and bar 75 cause the fluid to spread across the width of the recess 18, enabling the recess to be filled with fluid.

Covertile 80 in figures 11 (a) and (b), covertile 82 in figures12 (a) and (b), and covertile 84 in figures16 (a) and (b) operate in similar ways to those described above.

With the fill methods described above, it should be understood that the covertiles are generally 25 mm across, and the recess typically only 20-200 micrometres high. Overall fluid dispense volumes may be in the order of 20-300 microlitres.

Figure 13 (a) shows a covertile 90 having a body 91, legs 92 with fluid dispenser 100 dispensing fluid 102 onto the slide 1. In figure 13 (a), the fluid 102 has already been dispensed, and has formed a fluid reservoir in a fluid retention zone 97. The schematic figure shows a typical wicking pattern formed by the fluid as it contacts the covertile 90. In figure 13 (b), the fluid is just being dispensed onto the projection 94. In the volumes dispensed, the fluid forms a pool of comparable size to some of the covertile features. Not only does the fluid flow forward of the

covertile as shown in figure 13 (a), but it also flows under the covertile to at least partially fill recess 18. As mentioned above the fluid may be drawn into the recess further by movement of the covertile over the slide or suction applied to the rear of the recess 18.

Figure 14 (a) and (b) shows a further embodiment of a covertile 110 having a fluid receiving zone 118, a protruding section 114, and a nib 115. Fluid may be deposited directly on the nib 115 where it rolls over the protruding section 114 and under the covertile into fluid retention zone 117, and to the recess 18 as required. If fluid is placed too far ahead of the covertile, there are circumstances that may cause the fluid to reach the edge of the slide before wicking across the width of the recess 18. This can cause undesired wicking paths to form on the side of the slide. Figure 15 shows a cross section of the covertile, with the fluid being dispensed onto the nib 115. The nib and protruding section 114 provide a surface of around 4 mm long for depositing fluid. The nib provides an effective extension of the protruding surface for the dispensation of fluid. This is important where the slide may move on its mount, and given that the dispenser may only be able to be placed within 1mm or so of the desired position.

Figure 17 shows an example of how fluid spreads across a slide when deposited in front of a covertile 120.

In use, a covertile 10 is placed on a slide 1, as shown in figures 4, 5 and 6 to cover the sample 3. The slide 1 will typically be in a tray 21 as shown in figure 7, said tray 21 able to hold, for example, 10 slides and covertiles of the examples shown. The tray 21 may then be placed into a biological reaction apparatus, such as that disclosed in the provisional patent application by the same applicant filed on the same date, titled "Method and Apparatus for Providing a Reaction Chamber", filed on even date, the contents of which are hereby incorporated by reference.

Once the tray 21 is loaded into the apparatus (not shown) the slides 1 are held in position, typically at an angle of 5 degrees to the horizontal as shown schematically in figures 13 (b), 15 or 17. The covertile 10 is then moved by an arm (not shown) engaging the locating means 16. Typically, during a sequence

referred to as an "open fill", the covertile 10 is moved longitudinally along the surface of the slide 1 until the sample 3 is exposed. A fluid is then dispensed by a dispensing means 100 such as a probe attached to a pump, onto the fluid receiving zone 98 (as shown in figure 13 (b)). The amount of fluid dispensed is typically sufficient to fill the reaction chamber 24. The use of the covertile 10 with this fill mechanism or methodology allows a small volume of fluid to be uniformly distributed across the reaction chamber 24. Distributing the fluid across the reaction chamber 24 evenly and without bubbles or air spaces allows reactions to take place on the sample 3 with greater consistency. Also, dispensing fluid into an empty receiving zone where the reaction chamber already contains fluid causes the fluid within the chamber to be replaced by the fluid in the receiving zone minimising mixing of the fluid in the reaction chamber and newly dispensed fluid. The dimensions of the reaction provide a smooth flow of fluid from the reaction chamber such that there is little mixing of the fluids. This is advantageous as it allows a previous fluid to be replaced accurately, with minimal original fluid remaining to contaminate later fluids or reactions. This reduces the number of washes required to clear the reaction chamber 24.

The volume of fluid in a reaction chamber 24 may be, for example 150 microlitres or less, although volumes may vary depending on the application and the reaction chamber dimensions.

The reaction chamber 24 is able to retain fluid due to the surface tension of the fluid, unless additional fluid is added to the fluid receiving zone, or suction is applied (typically through reduces air pressure) at the end of the slide opposite the fluid receiving zone. The reaction chamber may be filled as it is formed by the covertile 10 being moved along the surface of the slide 1 to cover the sample holding region 52. Alternatively, the reaction chamber may be filled without the the covertile being moved relative to the slide, due to the process of capillary wicking of dispensed fluid into the reaction chamber.

In the present examples the covertile may be clamped to the slide when not in motion or retracted for an initial fill. The clamping mechanism (not shown) places

force around the edge of, for example, covertile 10 adjacent the lands 20 to locate the covertile 10 with respect to the slide 1 during a reaction.

During the withdrawal of the covertile 10 from the slide 1 it is sometimes desirable to remove the covertile from contact with the slide. In order to accomplish this, wings 26 engage the ramps 28 to lift the covertile clear of the slide. This causes the covertile 10 to lift off the slide 1 to prevent fluid contact between the slide 1 and covertile 10. In this way the slide can be cleared of virtually all fluid.

Parts of the covertile may have different material properties compared to the properties of the material of the covertile body, which is typically plastic. In one example (not shown) the recess may have different material properties, in order to provide a reaction chamber with certain material properties. A clear plastic material has been found to be suitable for the body of the covertile, to provide suitable mechanical properties such as reasonable strength and rigidity. The covertile needs to be sufficiently strong to be moved while clamping forces are applied to the covertile, as the clamping forces assist in providing a sealing surface between the lands of the covertile and the upper surface of the slide. The covertile may be moved to empty or fill the chamber, or also, to promote fluid movement within the reaction chamber to assist a reaction.

The covertile should ideally have some flexibility, as it is desirable that upon application of the clamp, the recessed face should deflect somewhat. This has been found to assist in moving the fluid within the reaction chamber and therefore increases the exposure of the sample to the fluid.

Other properties of the covertile include the ability to restrict the heat loss from the surface of the slide. Typically the slide will be mounted on a heated block, and the covertile will be placed over the sample o the slide. Heating the slide heats the sample and the fluid in the reaction chamber. If there is excessive heat loss from the covertile it is difficult to regulate the temperature of the fluid by heating the slide. Further, there may be an excessive temperature gradient across the reaction chamber, which is undesirable.

The recessed face 19, as shown in figure 2, may have different surface properties to the rest of the covertile. It has been found to be desirable to have similar material properties for the upper surface of the slide 2 and the recess 18. In one example, it is possible to coat the surface of the recess with a material, such as silicon dioxide. This coating may be approximately 110 nm thick. The coating provides a surface with material properties similar to that of a glass slide. It has also been found that there are benefits in applying a thin layer (for example 0.5-6nm) of Chromium Oxide ($Cr2O3$) to the recess before applying the silicon dioxide layer. This application of an intermediate layer between the silicon dioxide and plastic provides better adhesion and better thermal expansion properties for the recess. Further, coatings in general may be used to improve the flatness of the recess (which reduce nucleation sites and therefore bubble formation at high temperatures). The coatings may be used to modify the capillary flow characteristics of the fluid within the reaction chamber, create an impermeable barrier for gas or liquid between the covertile and fluid in the reaction chamber, or provide a chemically inert surface.

In another example, it is possible to replace the recessed face 18 with a glass insert supported by the plastic body of the covertile. It may also be possible to change the surface properties of the plastic by plasma discharge.

The covertiles shown in the examples may be used at temperatures approaching 100 degrees Celsius, especially when used for in-situ hybridisation reactions. At higher temperatures,

The claims defining the invention are as follows:

1 A covertile for a substrate containing a sample, comprising:

a side having a recess;

a fluid receiving zone in fluid communication with the recess; and

a locator such that the covertile may be controlled and located with respect to the substrate.

2 The covertile of claim1 wherein the fluid receiving zone is situated at a first end of the covertile.

3 The covertile of claim1 or 2 wherein the recess is a substantially flat portion recessed from a land portion.

4 The covertile of claim 3 wherein the land portion is located at the edge of the covertile, surrounding the recess on two sides.

5 The covertile of any of claims 1 to 4 wherein the locator is situated at a second end of the covertile remote from the first end.

6 The covertile of any of claims 1 to 5 wherein the covertile is made from a polymer material.

7 The covertile of any of claims 1 to 6 wherein the recess includes a coating of reduced surface roughness than the polymer material.

8 The covertile of claim7 herein the recess includes a coating with reduced porosity.

9 The covertile of claim 7 or 8 wherein the recess has one or more coatings.

10 The covertile of claims 9 wherein a first coating is a material having similar properties to the material of the slide.

11 The covertile of claim 10 wherein the first coating is silicon dioxide.

12 The covertile of claim 9 wherein a second coating is placed intermediate a first coating to provide improved contact properties between the covertile and first coating.

13 The covertile of any preceding claim wherein the width of the recess of the covertile is the no larger than the width of a microscope slide.

14 The covertile of any preceding claim wherein the fluid receiving zone includes projections around a fluid-receiving zone.

15 The covertile of claim 14 wherein two projections form a fluid retention zone at the fluid receiving zone.

DATED this 20th day of June, 2002

VISION BIOSYSTEMS LIMITED

By DAVIES COLLISON CAVE
Patent Attorneys for the applicant

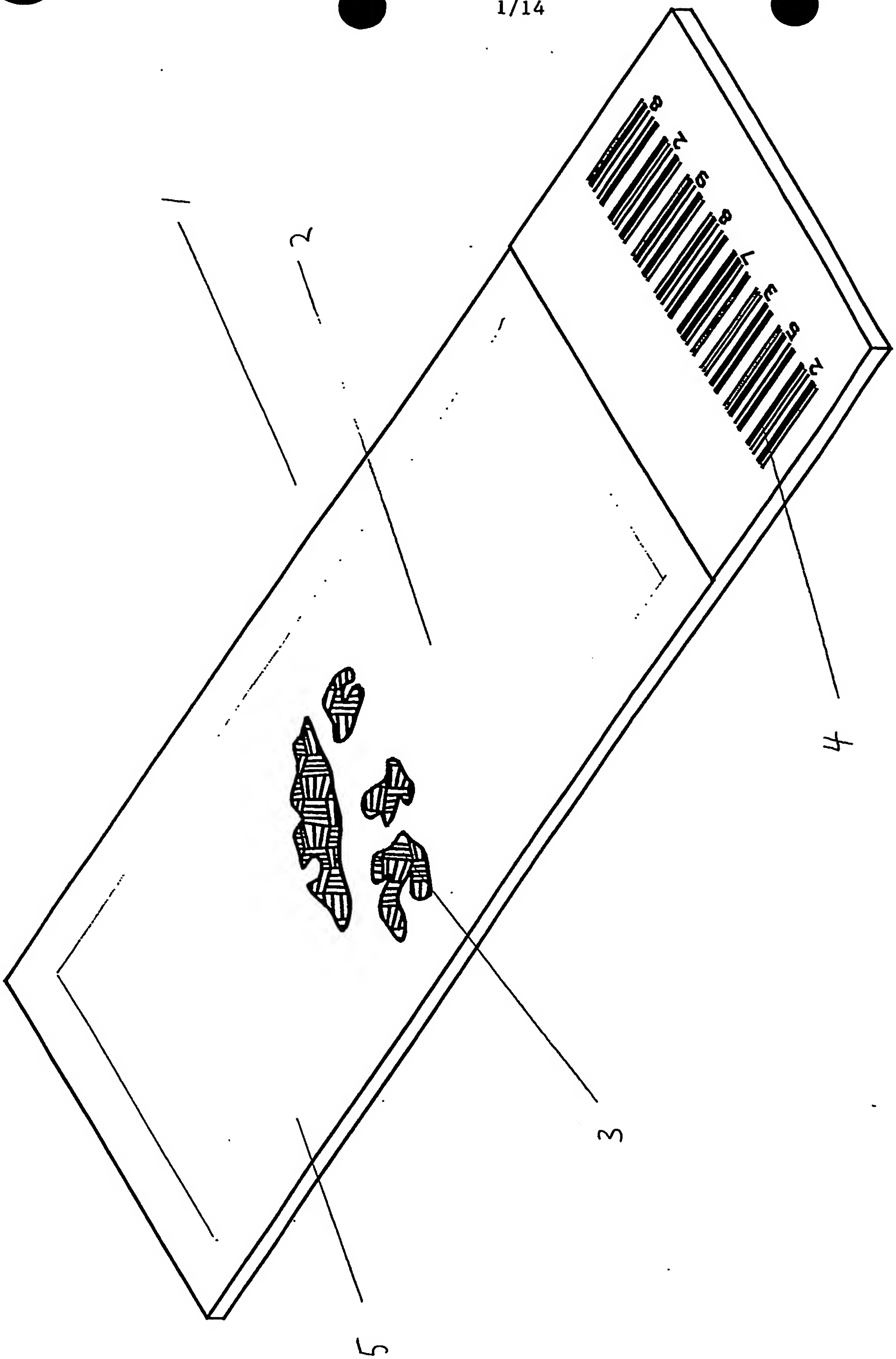


Figure 1

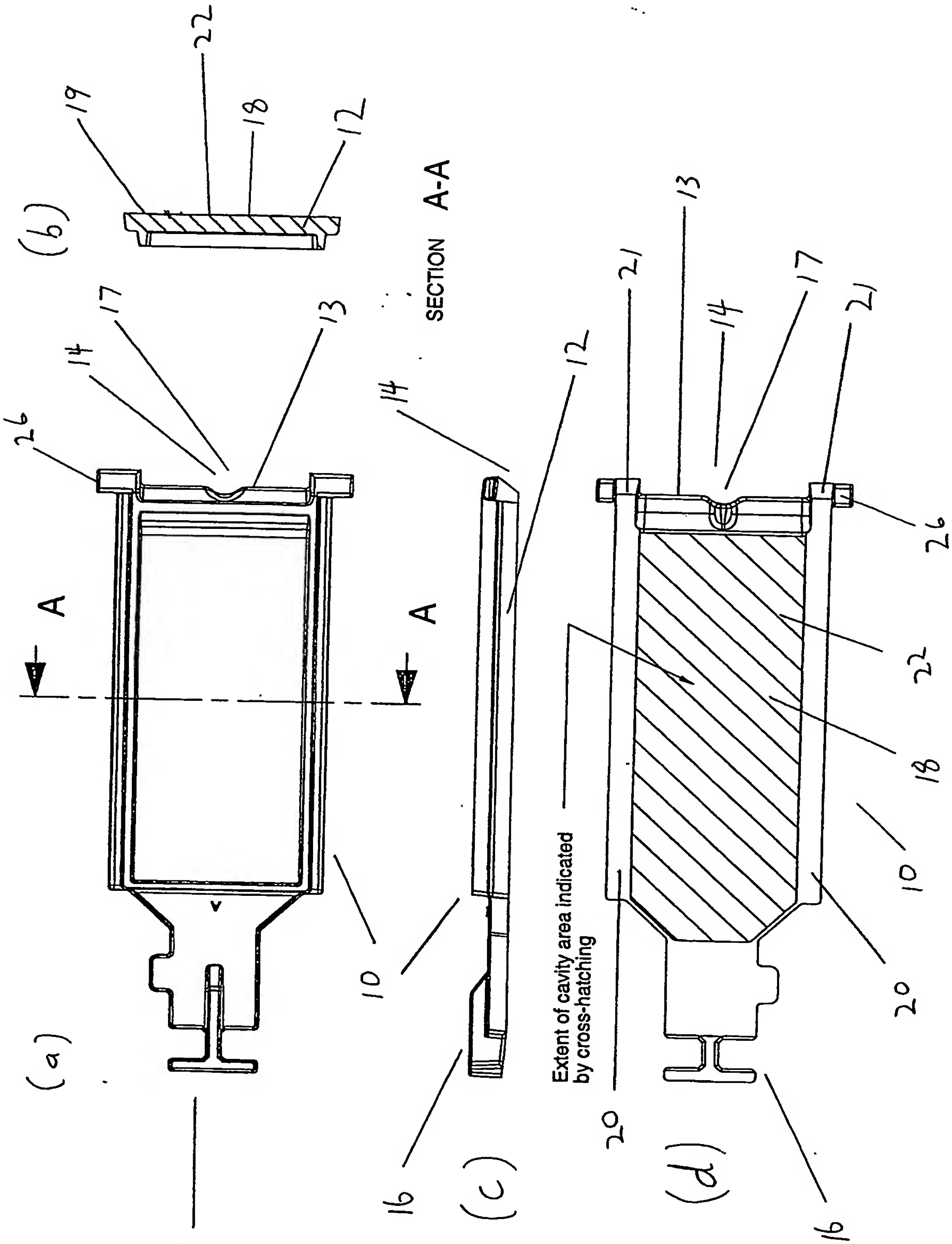

(a)
(b)
(c)
(d)
SECTION A-A
A
A
Extent of cavity area indicated by cross-hatching
10
12
13
14
16
17
18
19
20
21
22
26

Figure 2

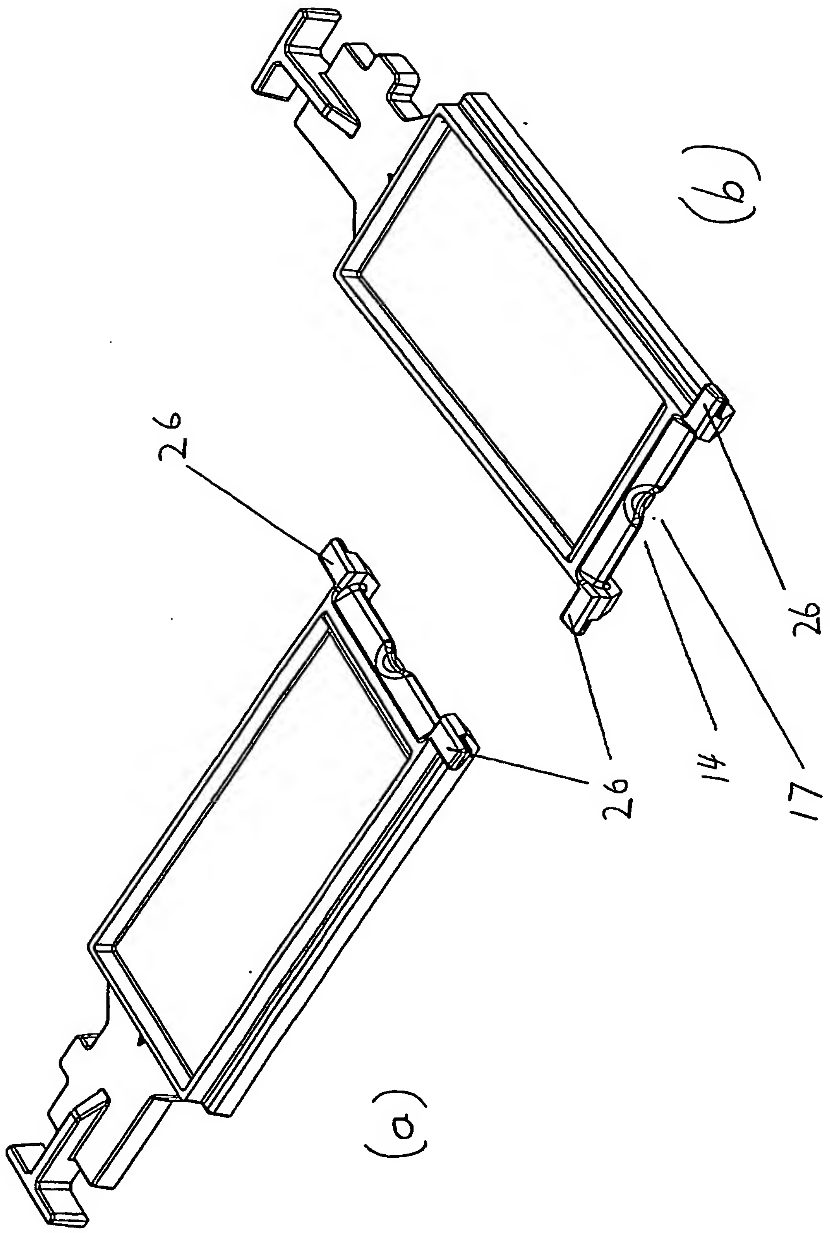


Figure 3

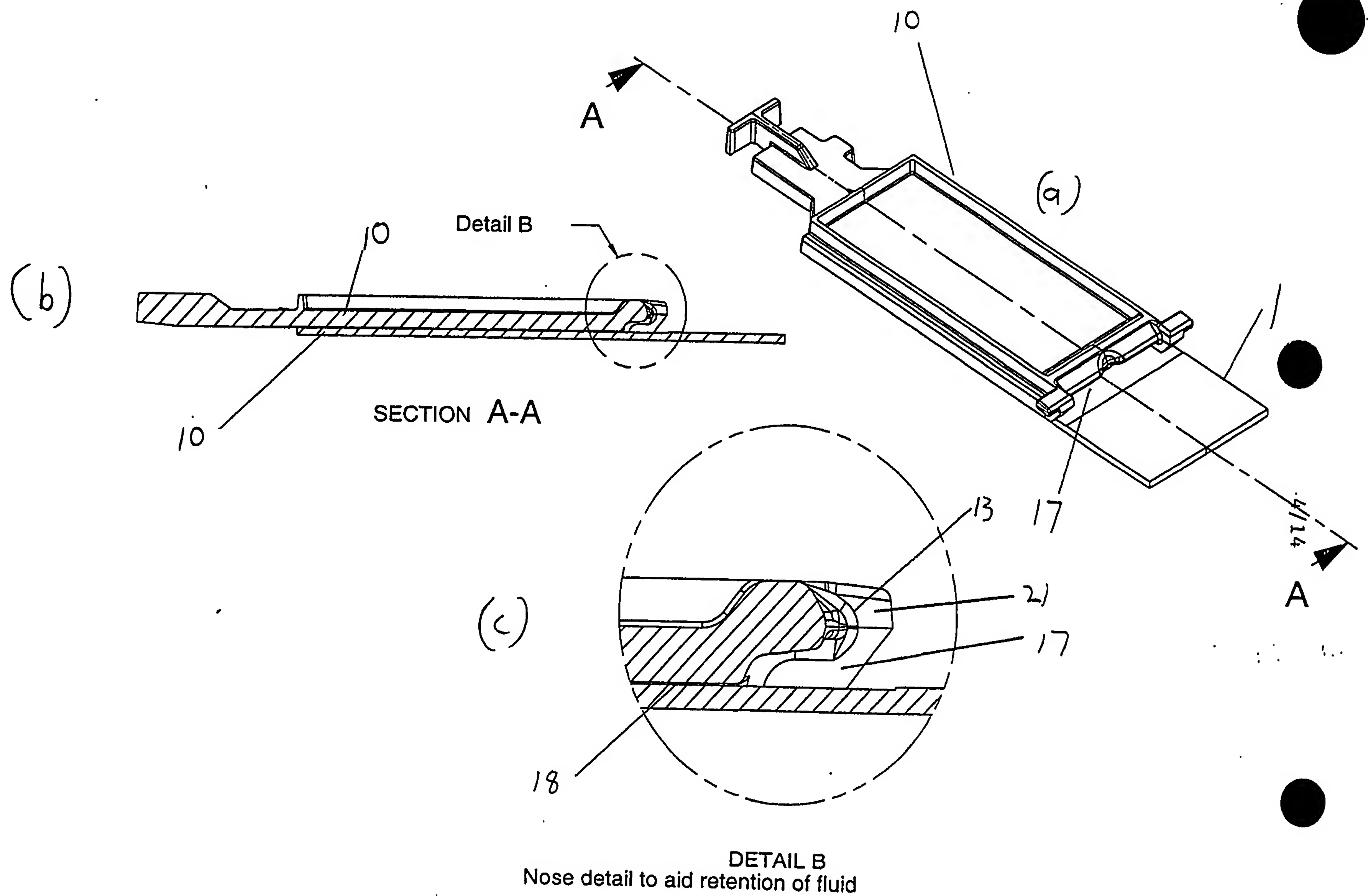

(a)
10
1
17
A
A
(b)
10
10
Detail B
SECTION A-A
(c)
13
21
17
18
DETAIL B
Nose detail to aid retention of fluid

Figure 4

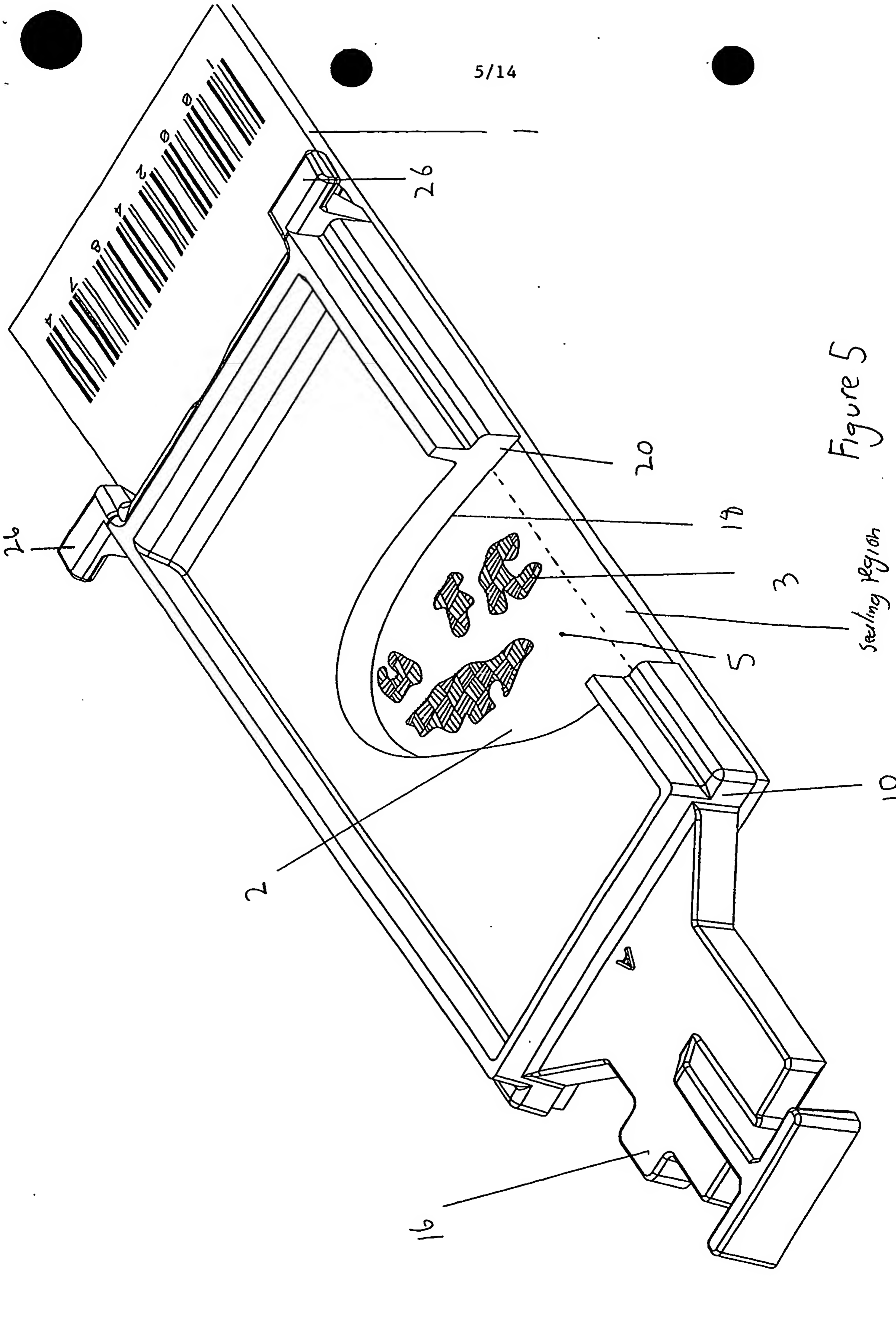


Figure 5

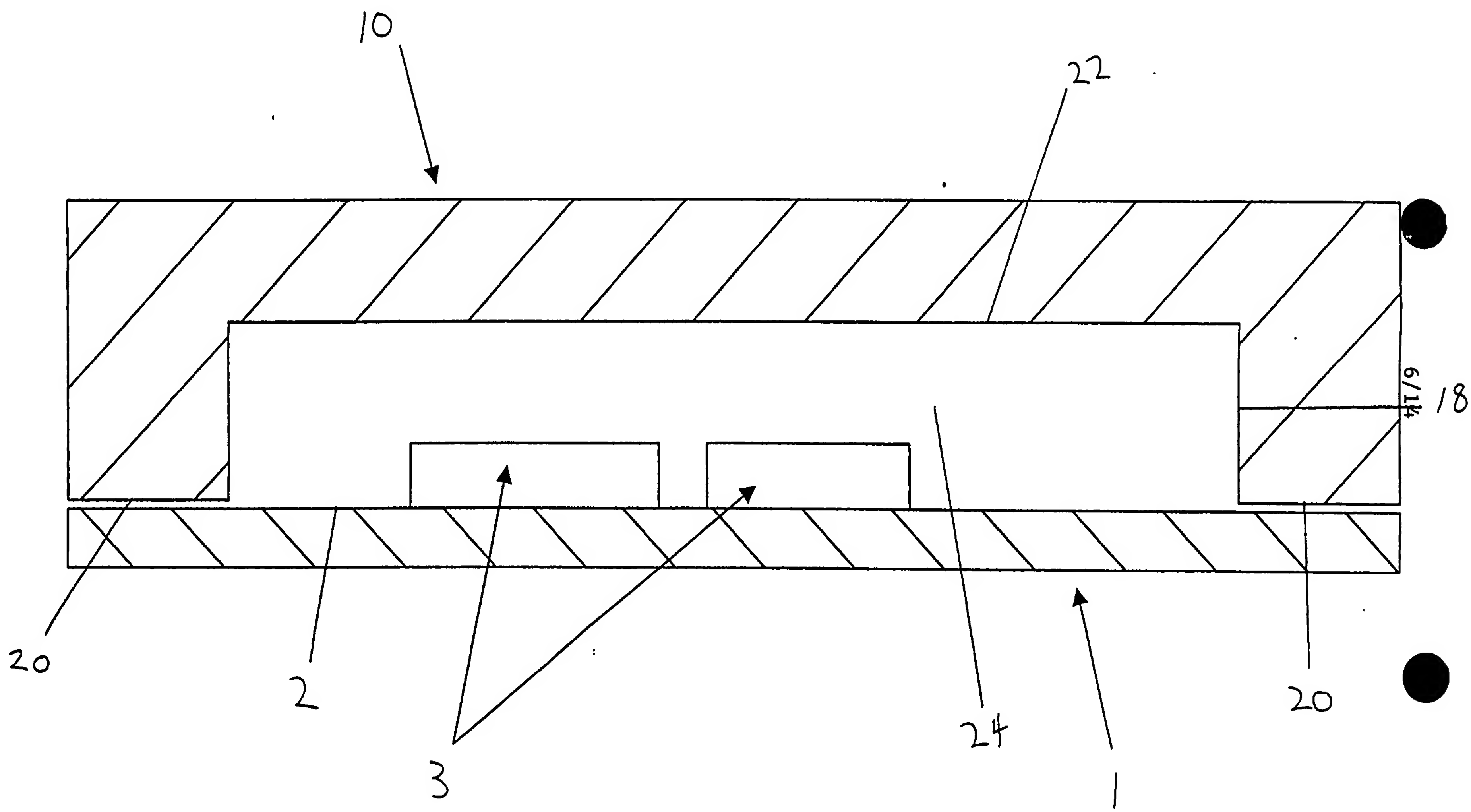


Figure 6

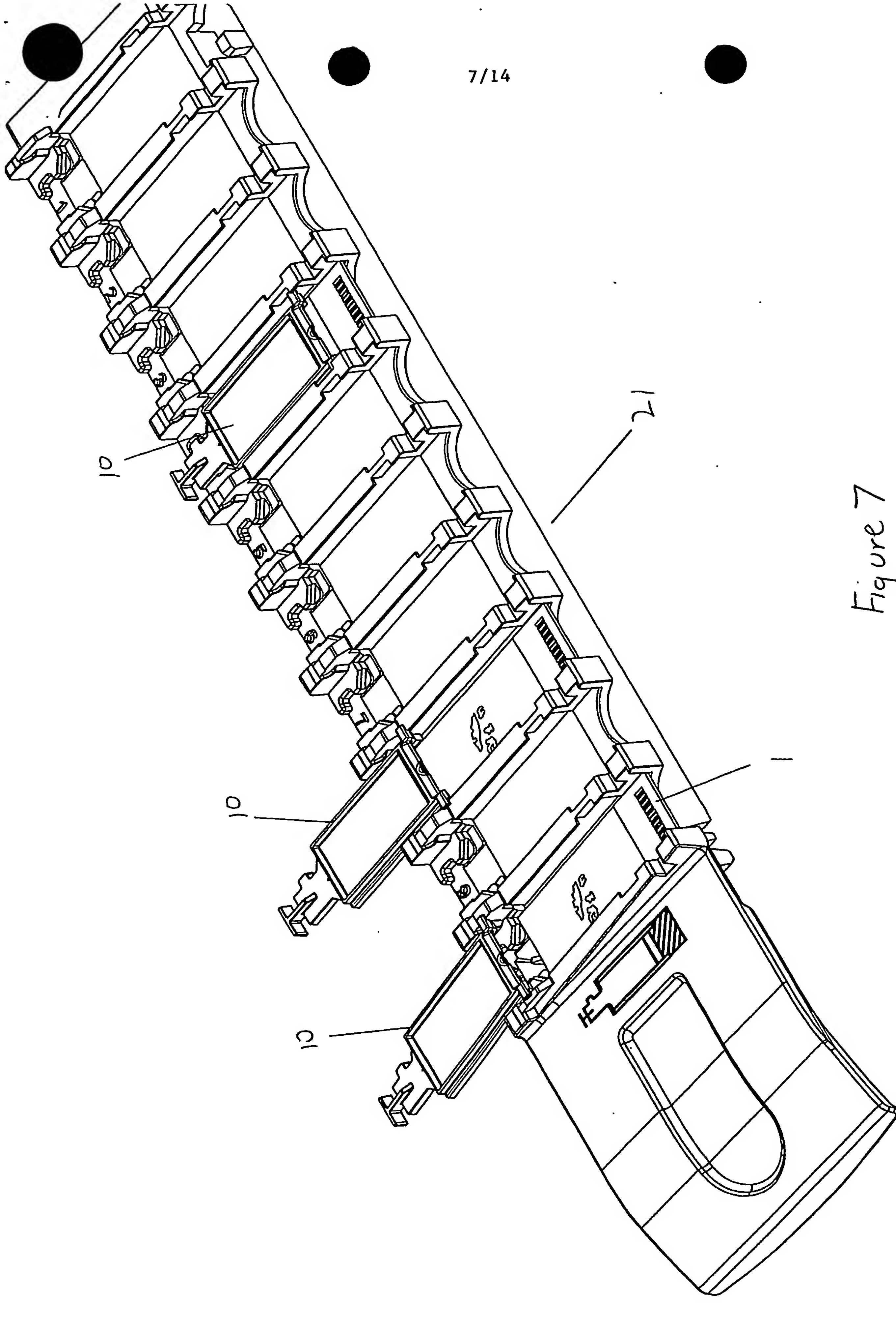
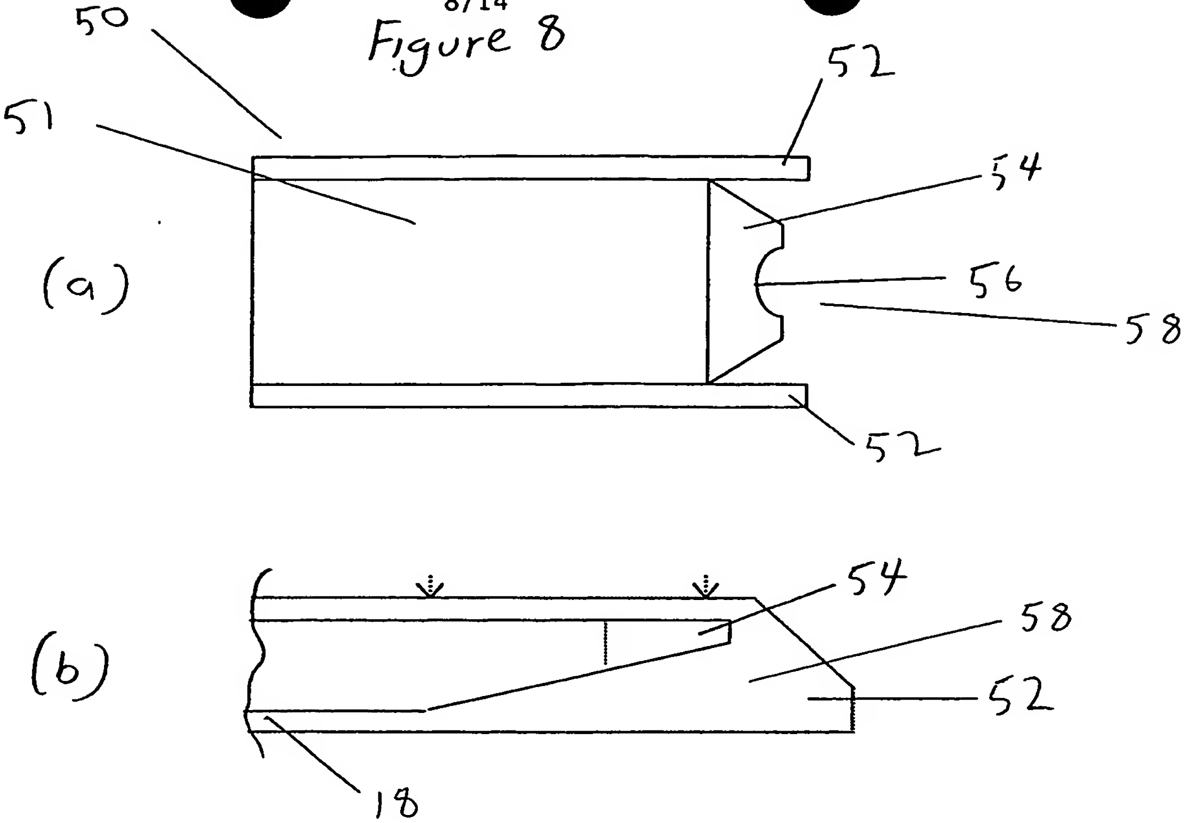


Figure 7

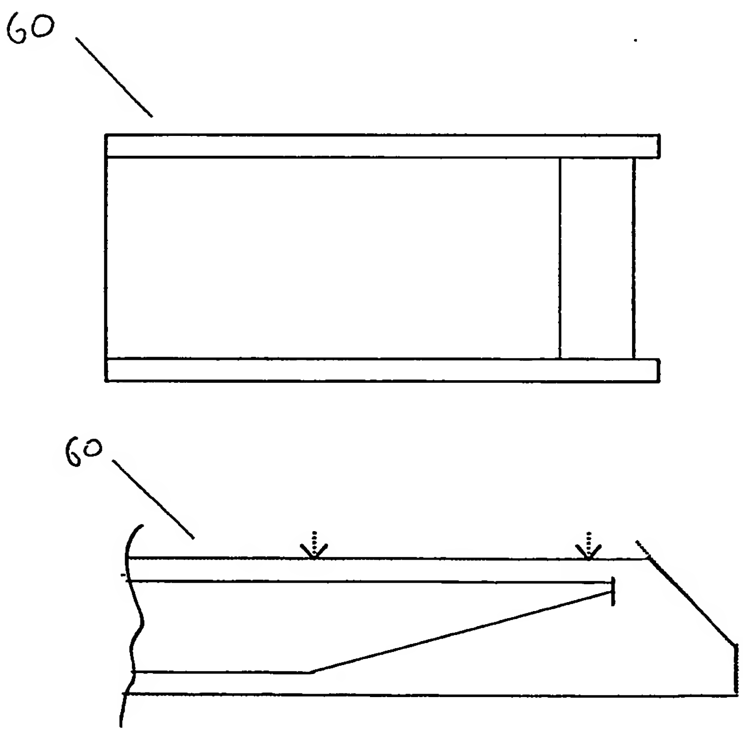
Figure 8



(a)

(b)

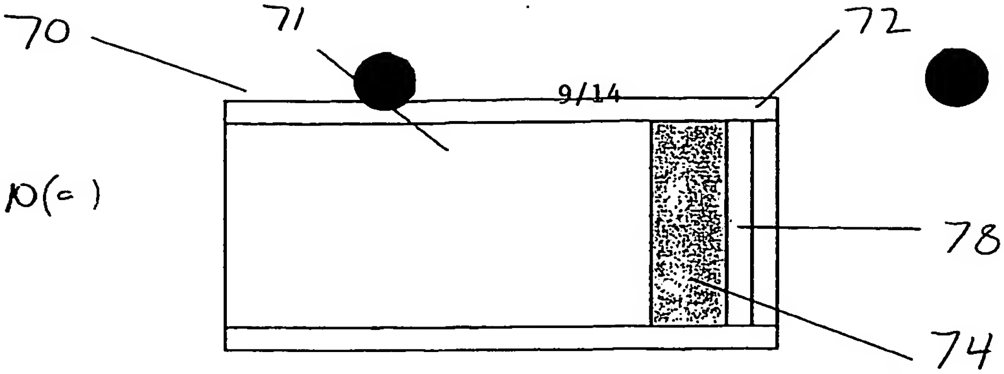
Figure 9



(a)

(b)

Figure 10(a)



(b)

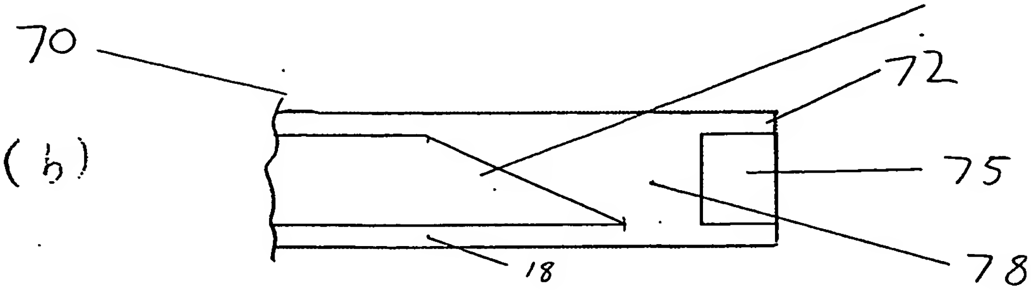
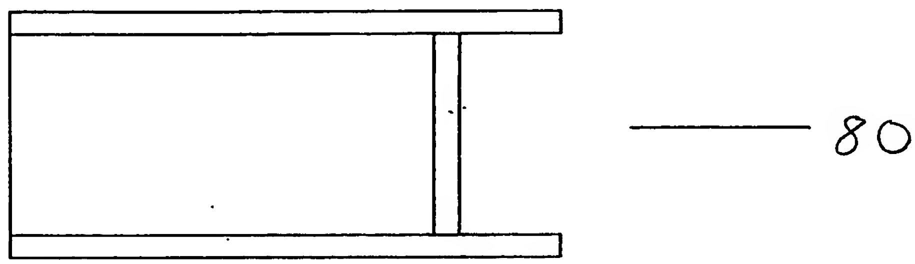


Figure 11(a)



(b)

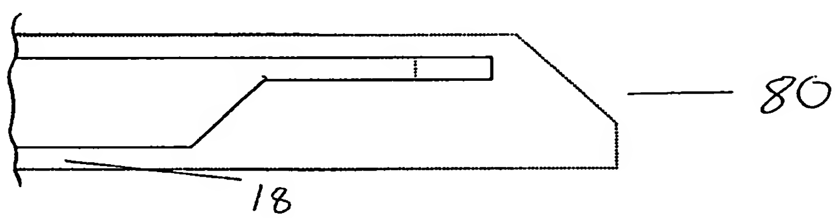
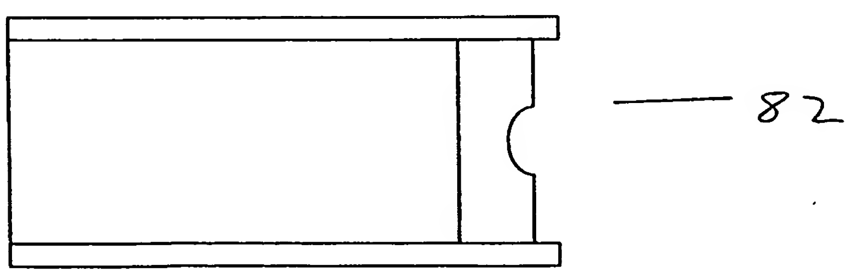
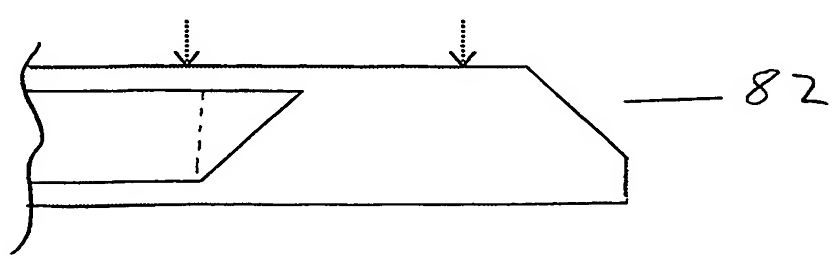
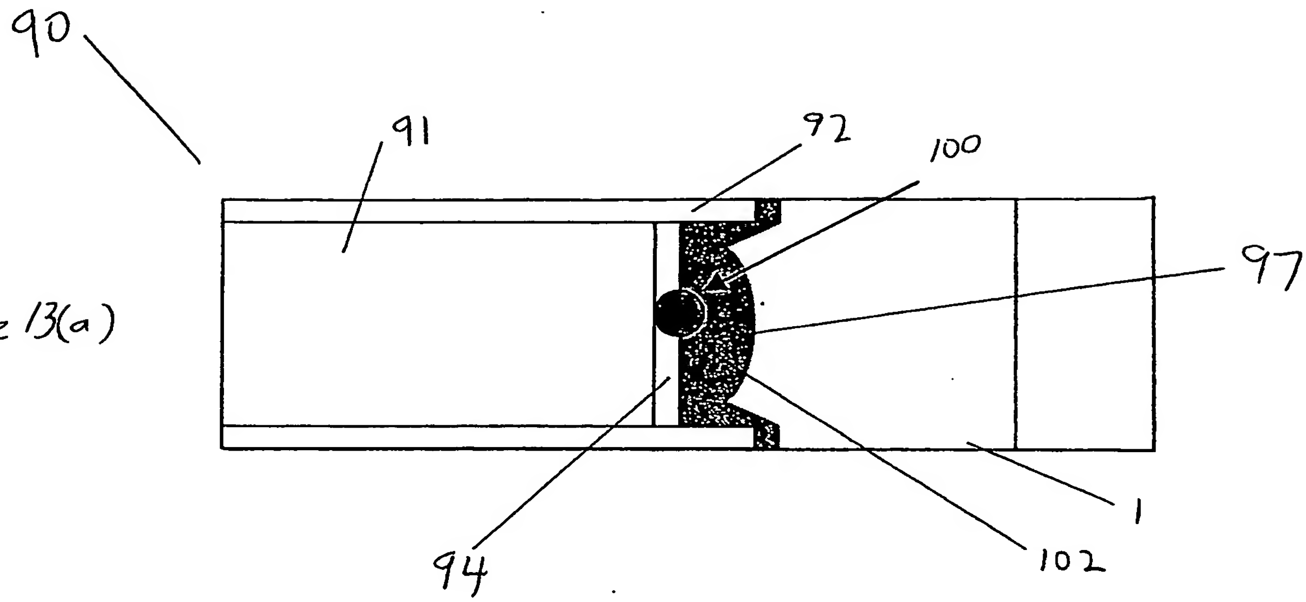


Figure 12(a)



(b)





gure 13(a)

Figure 13(b)

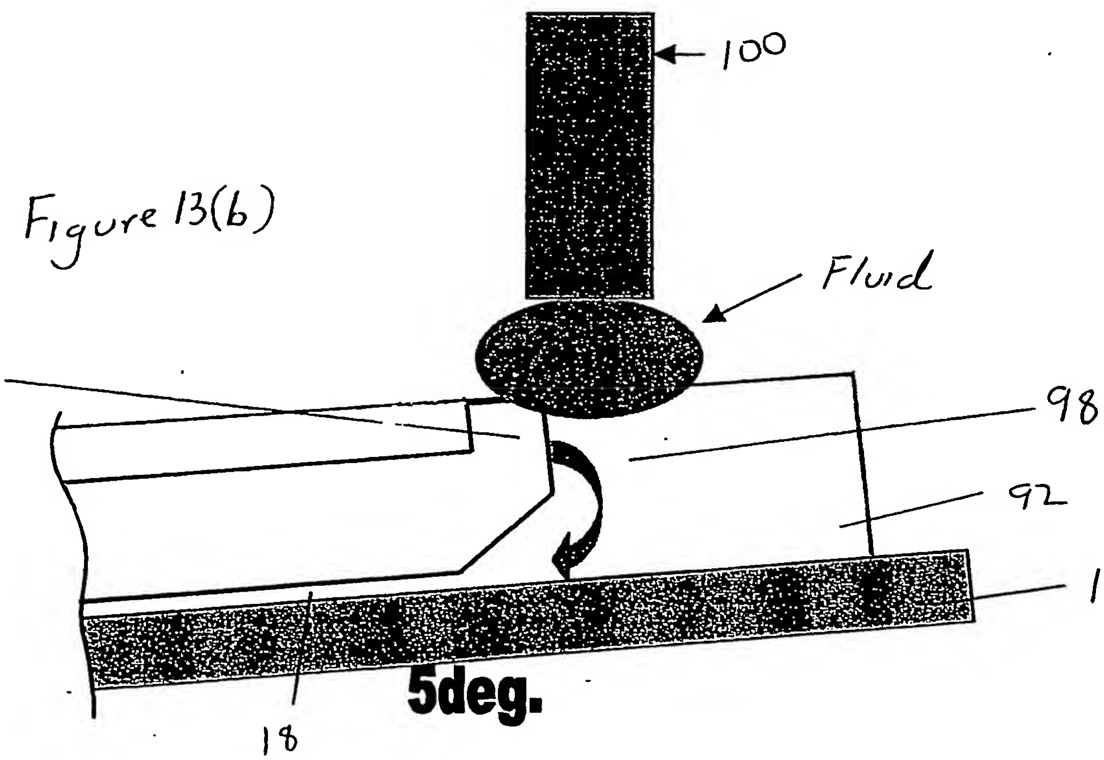


Figure 14

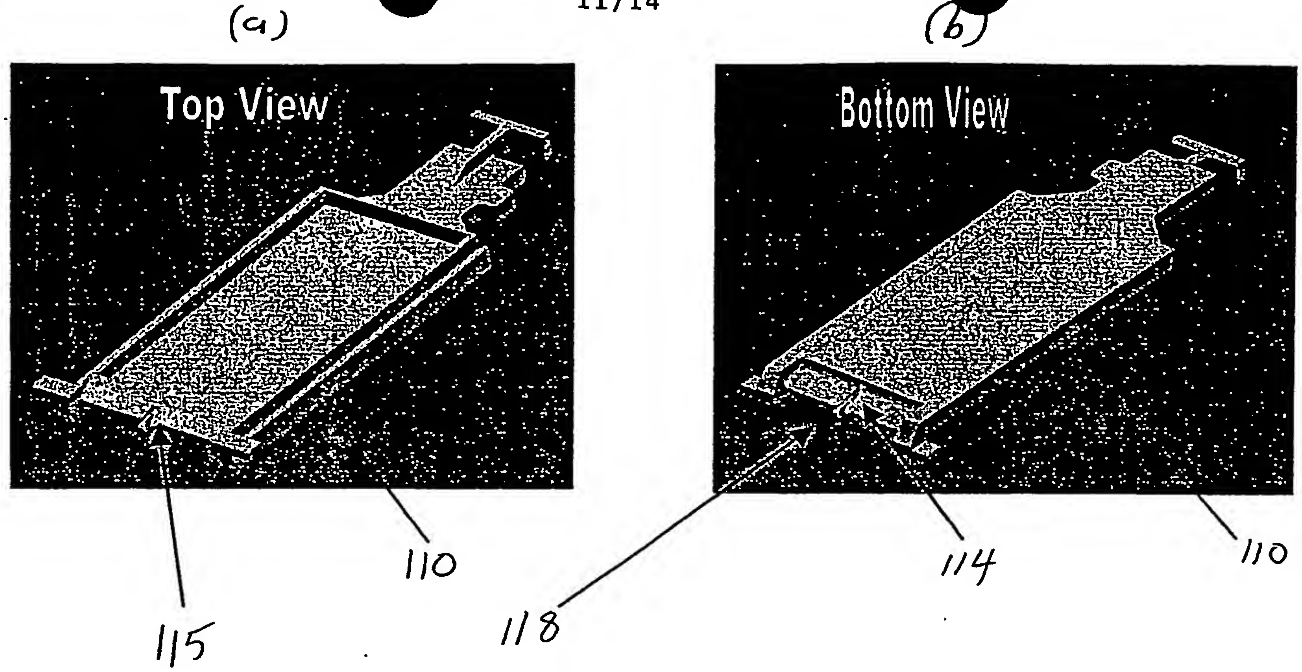


Figure 15

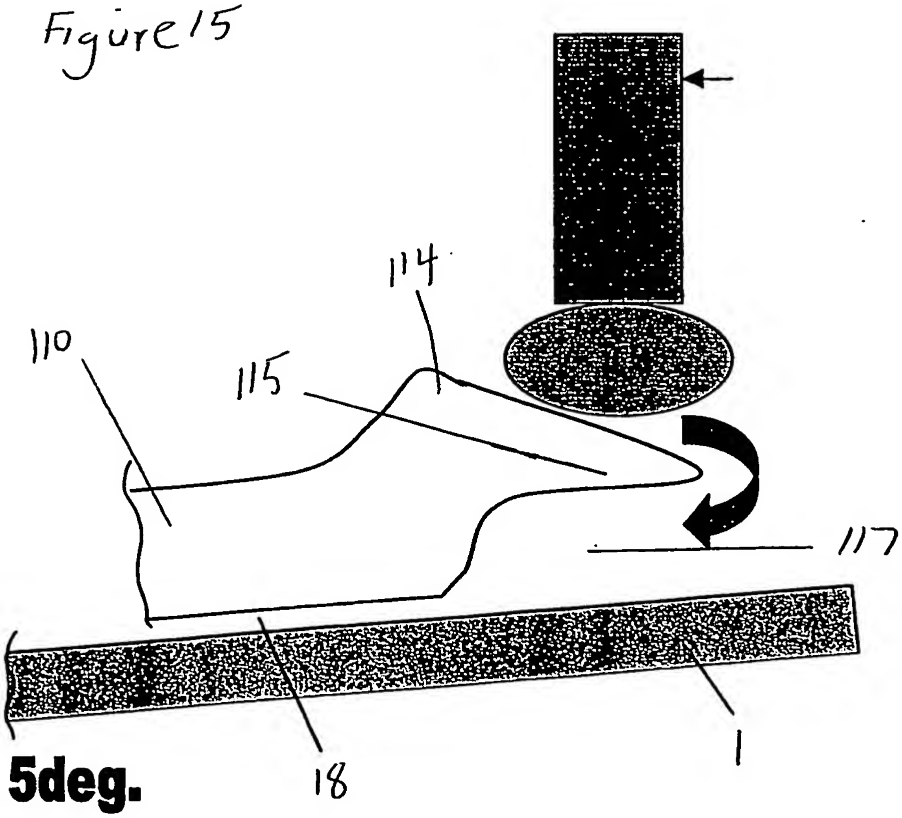
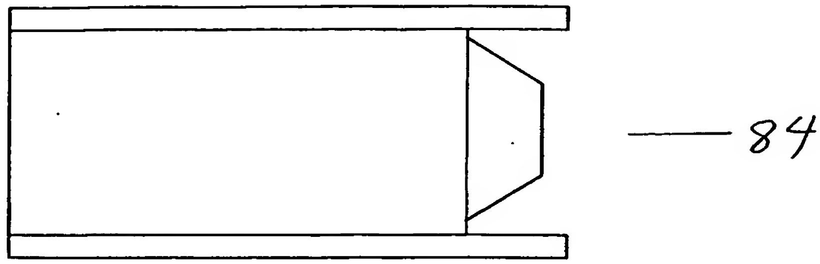


Figure 16

(a)



(b)

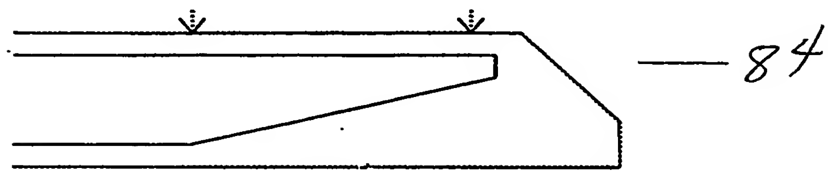
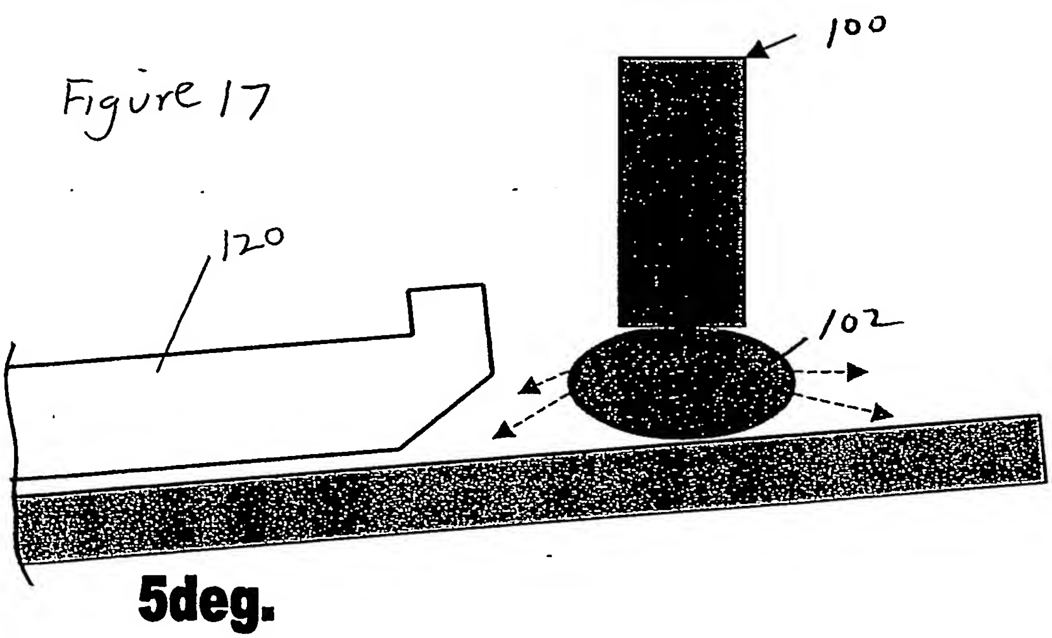


Figure 17



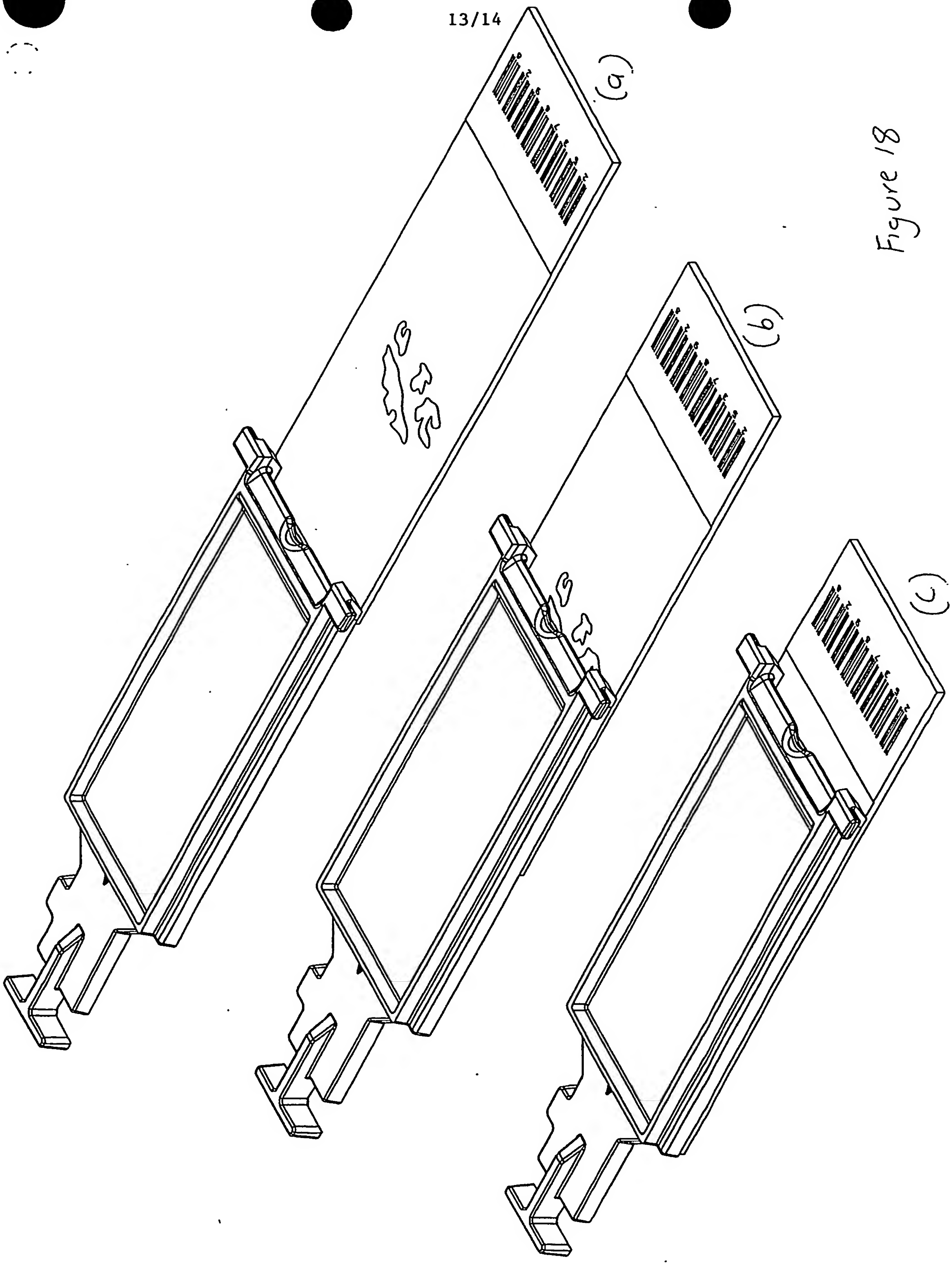


Figure 18

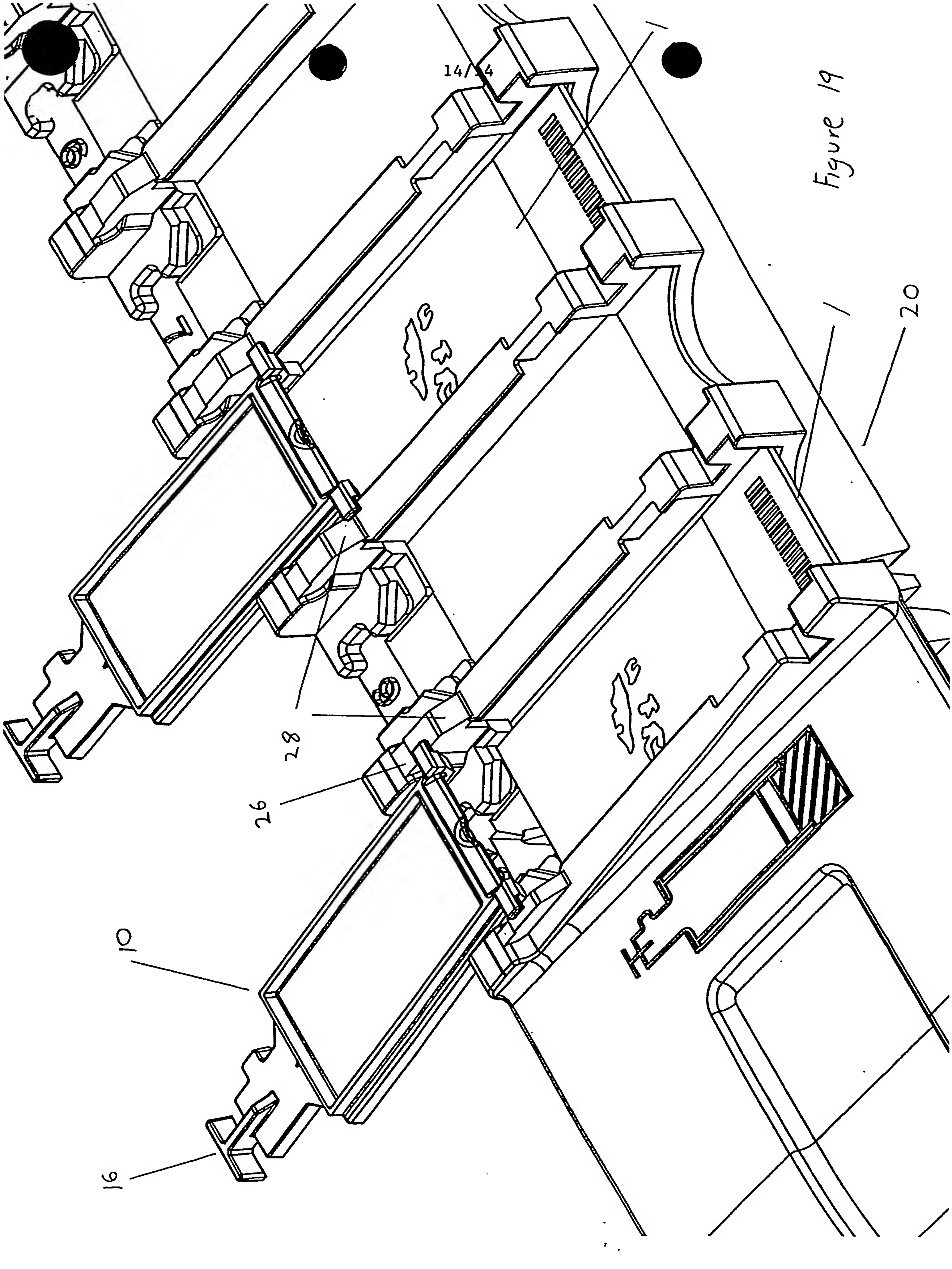


Figure 19